Mechanism of the stimulation of respiration by fatty acids in rat liver

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The mechanism of stimulation of hepatic respiration by fatty acids was studied in isolated rat hepatocytes. Stimulation of respiration by fatty acids varied from about 35% to about 105% depending on chain length. The stimulatory effect of octanoate (1 mM) or oleate (0.5 mM) was prevented by oligomycin (2 μg/ml). With carboxyatractyloside (100 μM) and ouabain (2 mM) the stimulation of respiration was partially inhibited (by 50–70 and 50–60%, respectively). From these results it can be concluded that the increased rate of respiration after addition of fatty acids is coupled to ATP synthesis. A large part (50–60%) of this ATP is utilized by the $(Na^+ + K^+)$-ATPase

Liver respiration *Fatty acid* *Liver energy metabolism* *(Hepatocyte)*

1. INTRODUCTION

Although stimulation of hepatic oxygen consumption by fatty acids is a widely recognized phenomenon (see [1]), little is known about the nature of the energy utilizing processes involved. Although some free fatty acids have been shown to have an uncoupler-like effect on isolated mitochondria [2–5], DeBeer et al. [6] concluded from experiments with oligomycin that in the intact perfused rat liver fatty acids do not uncouple mitochondrial oxidative phosphorylation. They suggested several futile cycles to explain the increased rate of respiration after the addition of fatty acids. Up to now, however, no evidence in favour of the existence of these futile cycles has been presented.

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In several recent studies on the effect of fatty acids on respiration in liver [7–10], the results of DeBeer et al. have largely been ignored. It was concluded that the stimulatory effect of fatty acids on hepatic respiration is not associated with an increased rate of ATP utilization, but with energy dissipation via reversal of the mitochondrial respiratory chain [9] or via uncoupling of mitochondrial oxidative phosphorylation [7].

In this study we show that the increased rate of respiration after the addition of fatty acids to isolated rat-liver cells is not due to an uncoupling-like effect, but to an increased rate of ATP utilization. Furthermore, we show that the increased rate of ATP utilization is in part due to activation of the $(Na^+ + K^+)$-ATPase in the plasma membrane.

2. MATERIALS AND METHODS

Rat-liver parenchymal cells were isolated from fed or 20–24 h starved male Wistar rats (200–300 g) by the method of Berry and Friend [11] with modifications [12]. Cells were preincubated at 37°C for 2 min with oligomycin or for 20 min in all other cases in Krebs-bicarbonate buf-

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fer containing 1.3 mM Ca^{2+} in closed plastic incubation vials with a gas phase of 95% O_2 and 5% CO_2. After the preincubation period, the cells were transferred to an oxygraph vessel equipped with a Clark-type O_2 electrode and thermostatically controlled at 37°C. After the rate of O_2 uptake had become constant, fatty acids were added. Samples of the cell suspension were also centrifuged through silicone oil (Wacker AR 200:Wacker AR 20 = 3:2, v/v) into ice-cold perchloric acid (final concentration 14% (w/v)) for measurement of adenine nucleotides, or into an ice-cold saturated mannitol solution for measurement of lactate dehydrogenase activity. The top layer was removed quickly and acidified with perchloric acid (final concentration 3.5% (w/v)) to measure extracellular adenine nucleotides or frozen in liquid nitrogen to measure lactate dehydrogenase activity. Adenine nucleotides and lactate dehydrogenase activity were measured according to [13].

All enzymes and biochemicals were obtained from Boehringer Mannheim (Mannheim, FRG). Bovine serum albumin (fraction V, Sigma, St. Louis, MO) was freed of fatty acids by the method of Chen [14]. All other chemicals were of analytical grade.

3. RESULTS

Fig.1 shows the stimulatory effect of octanoate on the respiration of rat-liver cells. The stimulation is completely oligomycin-sensitive (cf. [6]). Respiration was stimulated again when after oligomycin the uncoupler 2,4-dinitrophenol was added (fig.1). A similar stimulation was obtained with butanoate (55%), pentanoate (55%), hexanoate (85%), decanoate (95%), dodecanoate (105%), palmitate (35%) and oleate (35%) (not shown).

In fig.2 titration experiments with oligomycin, carboxyatractyloside and ouabain are shown, in which the effect of the 3 inhibitors was investigated on both basal and octanoate-stimulated respiration in suspensions of isolated hepatocytes from fasted rats. Oligomycin inhibited the basal rate of oxygen uptake maximally at the lowest concentration used (2 μg/ml). This concentration of oligomycin also prevented the stimulation of respiration by 1 mM octanoate. In contrast, the stimulatory effect of octanoate was not completely sensitive to carboxyatractyloside; the stimulation in the presence of 200 μM carboxyatractyloside was 35% of that in the absence of the inhibitor. Similarly, with 5 mM ouabain 40% of the stimulatory effect of octanoate observed without inhibitor remained.

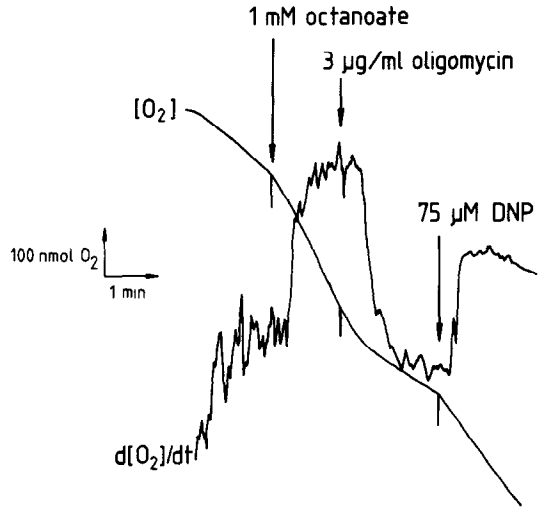

Fig.1. Effect of the successive addition of octanoate, oligomycin and 2,4-dinitrophenol (DNP) on O_2 uptake in rat-liver cells. Isolated liver cells from fasted rats were preincubated as described in section 2. In the oxygraph vessel the oxygen concentration ([O_2]) and rate of O_2 uptake (d[O_2]/dt) were monitored. A representative experiment is shown.

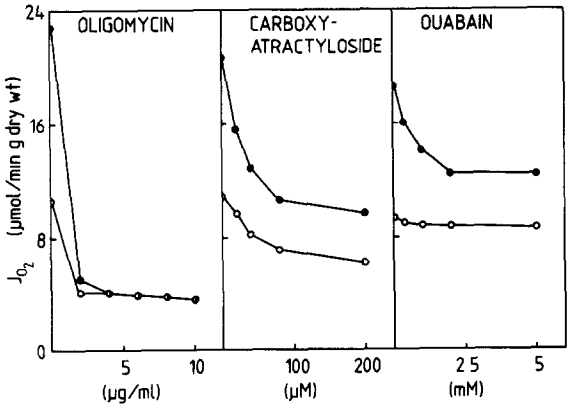

Fig.2. Effect of different concentrations of oligomycin, carboxyatractyloside and ouabain on respiration in isolated rat-liver cells in the absence and presence of octanoate. Liver cells isolated from fasted rats were preincubated as described in section 2. The rate of oxygen uptake was measured in the absence (○) and presence (●) of 1 mM octanoate.

Table 1

The effects of oligomycin, carboxyatractyloside and ouabain on stimulation of oxygen uptake by fatty acids

Additions	O_2 uptake (μmol/min per g dry wt) in liver cells from					
	Fasted rats			Fed rats		
	Control	+ octanoate (1 mM)	+ oleate (0.5 mM)	Control	+ octanoate (1 mM)	+ oleate (0.5 mM)
None	11.7 ± 0.2 (14)	20.7 ± 1.0 (8)	16.1 ± 0.4 (6)	10.8 ± 0.5 (10)	18.2 ± 0.6 (8)	15.9 ± 1.3 (3)
Oligomycin (2 μg/ml)	5.7 ± 0.2 (6)	5.9 ± 0.3 (3)	5.6 ± 0.4 (6)	6.4 ± 0.3 (5)	6.2 ± 0.7 (7)	6.6 ± 0.6 (3)
Carboxyatractyloside (100 μM)	5.4 ± 0.4 (11)	9.0 ± 0.6 (6)	7.5 ± 0.4 (5)	5.2 ± 0.3 (7)	8.1 ± 0.2 (5)	6.7 ± 0.6 (2)
Ouabain (2 mM)	8.9 ± 0.7 (4)	13.9 ± 0.1 (3)	8.8	8.5 ± 0.2 (3)	12.3 ± 0.2 (3)	10.6

Cells were preincubated as described in section 2. Rates of O_2 uptake are given as means ± SE (with the number of different cell preparations used given in parentheses). Oleate was dissolved in bovine serum albumin, resulting in a final albumin concentration of 1% (w/v). Bovine serum albumin alone stimulated respiration less than 5%

From table 1 it can be seen that the complete inhibition by oligomycin and the partial inhibition by carboxyatractyloside (50–70%) and ouabain (50–60%) were observed not only when octanoate was used to stimulate respiration but also when oleate was used. Moreover, similar results were obtained with suspensions of hepatocytes isolated either from fasted rats or from fed rats.

All concentrations of oligomycin used strongly lowered the cellular content of ATP, but at the same time the ADP and AMP content increased, so that the total cellular content of adenine nucleotides remained constant (fig.3). The extracellular amount of adenine nucleotides was always lower than 5% of the total amount (not shown). The leak of lactate dehydrogenase activity from the cells was never higher than 10% of the total activity in the suspensions (not shown).

4. DISCUSSION

It is generally accepted that addition of a respiratory substrate can only lead to increased rates of respiration if a demand for ATP already exists or is evoked by this addition. However, the stimulation of hepatic respiration by fatty acids has up to now been only partially attributable (30–70%, depending upon the precise experimental conditions) to an increased rate of ATP utilization for biosynthetic processes, such as gluconeogenesis and ureogenesis [6,7,9].

If fatty acids stimulate respiration in intact cells via an uncoupling-like effect [7], one would not expect the stimulatory effect to be sensitive to inhibitors such as oligomycin and carboxyatractyloside, as was observed in our experiments (figs 1,2 and table 1; see also [6]).

Interference with cellular ATP synthesis (e.g. by adding oligomycin) can under some conditions lead to cell damage [15]. Since the cellular content of total adenine nucleotides (ATP + ADP + AMP) was maintained (fig.3) and leakage of lactate dehydrogenase activity from the cells was low (not shown), this cannot explain the failure of fatty acids to stimulate respiration in the presence of oligomycin.

As pointed out by Scholz et al. [7], a significant contribution of peroxisomal β-oxidation can be excluded. For instance, induction of peroxisomal fatty acid oxidation enzymes by ciprofibrate had no effect on the magnitude of the stimulation [7].

The conclusion is therefore that the increased rate of respiration is, indeed, coupled to ATP synthesis. The experiments with carboxyatractyloside (fig.2 and table 1) show that the increased rate of ATP utilization is in part localized inside the

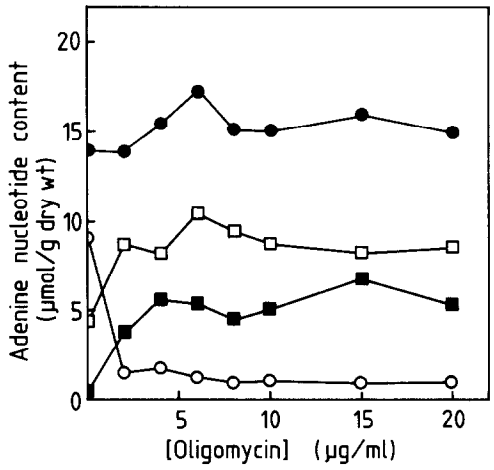

Fig.3. Effect of oligomycin on cellular adenine nucleotides. Rat-liver cells isolated from fasted rats were preincubated for 2 min and intracellular adenine nucleotides were measured as described in section 2. (●) ATP + ADP + AMP, (○) ATP, (□) ADP, (■) AMP. A representative experiment is shown.

mitochondrial matrix (30–50%) and that the remaining part (50–70%) is localized in the extramitochondrial compartments.

The nature of the ATP utilizing process in the cytosol became clear when it was found that the stimulation of respiration by octanoate was decreased by ouabain, an inhibitor of the $(Na^+ + K^+)$-ATPase of the plasma membrane. This inhibition by ouabain was approximately equal to that by carboxyatractyloside (table 1), indicating that the $(Na^+ + K^+)$-ATPase is the major ATP-utilizing process in the cytosol that is stimulated by fatty acids. The mechanism of this stimulation is at present unclear.

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